EXTENDED REPORT

Comparison of in vitro-specific blood tests with tuberculin skin test for diagnosis of latent tuberculosis before anti-TNF therapy

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Introduction: Latent tuberculosis infection (LTBI) is detected with the tuberculin skin test (TST) before anti-TNF therapy. We aimed to investigate in vitro blood assays with TB-specific antigens (CFP-10, ESAT-6), in immune-mediated inflammatory diseases (IMID) for LTBI screening.

Patients and methods: Sixty-eight IMID patients with (n = 35) or without (n = 33) LTBI according to clinico-radiographic findings or TST results (10 mm cutoff value) underwent cell proliferation assessed by thymidine incorporation and PKH-26 dilution assays, and IFNγ-release enzyme-linked immunosorbent spot (ELISPOT) assays with TB-specific antigens.

Results: In vitro blood assays gave higher positive results in patients with LTBI than without ($p<0.05$), with some variations between tests. Among the 13 patients with LTBI diagnosed independently of TST results, 5 had a negative TST (38.5%) and only 2 a negative blood assays result (15.4%). The 5 LTBI patients with negative TST results all had positive blood assays results. Ten patients without LTBI but with intermediate TST results (6–10 mm) had no different result than patients with TST result $\leqslant 5$ mm ($p>0.3$) and lower results than those with LTBI ($p<0.05$) on CFP-10+ESAT-6 ELISPOT and CFP-10 proliferation assays.

Conclusion: Anti-TB blood assays are beneficial for LTBI diagnosis in IMID. Compared with TST, they show a better sensitivity, as seen by positive results in 5 patients with certain LTBI and negative TST, and better specificity, as seen by negative results in most patients with intermediate TST as the only criteria of LTBI. In the absence of clinico-radiographic findings for LTBI, blood assays could replace TST for antibiotherapy decision before anti-TNF.

TNFα blocker agents are approved for the treatment of immune-mediated inflammatory diseases (IMID) and provide marked clinical benefit. However, they can reactivate tuberculosis (TB) infection in patients previously exposed to TB bacilli.[1 2] The presence of quiescent mycobacteria defines latent TB infection (LTBI).[3 4] Thus, screening for LTBI is necessary before initiating therapy with TNF blockers.[5] However, to date, no perfect gold standard exists for detecting LTBI, and tuberculin skin test (TST) remains largely used.

The recommendations for detecting LTBI differ worldwide.[3 6 7] In France, recommendations were established in 2002 by the RATIO (Research Axed on Tolerance of Biotherapies) study group for the Agence Française de Sécurité Sanitaire des Produits de Santé.[8 9] Patients are considered to have LTBI requiring treatment with prophylactic antibiotics before starting anti-TNFα therapy if they had previous TB with no adequate treatment, tuberculosis primo-infection, residual nodular tuberculous lesions larger than 1 cm^3 or old lesions suggesting TB diagnosis (parenchymatous abnormalities or pleural thickening) as seen on chest radiography or weals larger than 10 mm in diameter in response to the TST. Adequate anti-TB treatment was defined as treatment initiated after 1970, lasting at least 6 months and including at least 2 months with the combination rifampicin–pyrazinamide. The choice of the threshold of 10 mm for the TST result was established in 2002 in France since the programme of vaccination with bacille Calmette–Guérin (BCG) was mandated in France, and nearly 100% of the population has been vaccinated. Nevertheless, after July 2005, the threshold was decreased to 5 mm as in most of all other countries.[10]

The TST is the current method to detect LTBI but has numerous drawbacks. Indeed, the TST requires a return visit for reading the test result. It has a poor specificity, since previous BCG vaccination and environmental mycobacterial exposure can result in false-positive results in all subjects.[6 11 12] This poor specificity can lead to unnecessary treatment with antibiotics, with a significant risk of drug toxicity.[13–15] On the other hand, TST in IMID may often give a more negative reaction than in the general population, mainly because of the disease or immunosuppressive drug use.[16 17] This poor sensitivity can lead to false-negative results, with a subsequent risk of TB reactivation with anti-TNF therapy.

The identification of genes in the mycobacterium TB genome that are absent in BCG and most environmental mycobacteria offers an opportunity to develop more specific tests to investigate *Mycobacterium tuberculosis* (*M. tuberculosis*) infection, particularly LTBI.[18] Culture fibrate protein-10 (CFP-10) and early secretory antigen target-6 (ESAT-6) are two such gene products that are strong targets of the cellular immune response in TB patients. In vivo-specific T-cell based assay

Abbreviations: BCG, bacille Calmette–Guérin; CFP-10, culture fibrate protein-10; ELISPOT, enzyme-linked immunosorbent spot; ESAT-6, early secretory antigen target-6; IFNγ, interferon gamma; IMID, immune-mediated inflammatory diseases; LTBI, Latent tuberculosis infection; TB, tuberculosis; TST, tuberculin skin test investigating interferon gamma (IFNγ) release or T-cell proliferation in the presence of these specific mycobacterial antigens could be useful in screening for LTBI before anti-TNF therapy. New IFNγ-based ex vivo assays involving CFP-10 and ESAT-6 (T-SPOT TB, Oxford Immunotec, Abingdon, UK) and QuantiFERON TB Gold (QFT-G; Cellestis, Carnegie, Australia) allow for diagnosis of active TB, recent primo-infection or LTBI.[12] These tests seem to be more accurate than the TST for this purpose in the general population.[12] To date, the performance of the commercial assays in detecting LTBI in patients with IMID receiving immunosuppressive drugs has not been demonstrated, and the frequency of indeterminate results is still debated.[19–21]

We aimed to investigate the performance of homemade anti-CFP-10 and anti-ESAT-6 proliferative and enzyme-linked immunosorbent spot (ELISPOT) assays in detecting LTBI in patients with IMID before anti-TNFα therapy. We analysed two subgroups of patients: those with confirmed LTBI independent of TST result, and those with LTBI based exclusively on a positive TST result between 6 and 10 mm.

PATIENTS AND METHODS

Patients

Between April 2003 and May 2005, blood samples were taken from 68 patients with rheumatoid arthritis (RA) (n = 29), spondylarthropathy (n = 25) or Crohn's disease (n = 14) before they began anti-TNF therapy. All patients fulfilled recognised international criteria for these diseases.[22 23] Some patients were also included in a previous published longitudinal study investigating alteration of mycobacterial-specific immune response on treatment with anti-TNFα.[24] Patients were divided in 2 groups according to absence (group I, n = 33) or presence (group II, n = 35) of LTBI defined as above with TST result ⩽10 mm because they were included before July 2005, after which the cutoff level was changed. Characteristics of patients are shown in table 1. No patient had active TB infection. All patients had not received anti-TNF treatment or other biotherapy, and all had been vaccinated with BCG in childhood. Patients gave their informed consent, and the study was approved by the local ethics committee.

TST procedure

The 68 patients underwent the TST the same day as the blood sample was taken, with 5 tuberculin units corresponding to 0.1 ml of purified protein derivative (PPD) (Sanofi Pasteur, France), according to the intradermal Mantoux method: 72 h after inoculation, the main diameter of skin induration was recorded (in millimetres) by an experienced examiner. Positivity or negativity according to the 10-mm threshold of induration was reported in all patients, and the exact size of the diameter was noted for 51 patients (23 in group I and 28 in group II) (table 1). Among the 23 patients of group I with a recorded test result, 10 had a result between 6 and 10 mm and thus would have been included in group II according to the modification of the French recommendations after July 2005. Among group II patients, 13 had a proven LTBI on the basis of clinical or radiographic findings, independent of the TST result. Among them, 5 had a TST result <10 mm. Two of these 5 patients (40%) received prednisone less than 10 mg/day, and 2 (40%) received oral methotrexate.

In vitro assays

After isolation of peripheral blood mononuclear cells according to standard procedures using Ficoll gradient, we performed thymidine incorporation (studied after 5 days of culture) and IFNγ-release ELISPOT assays (studied after 18 h of culture of 2×10^5 cells per well) in the presence of recombinant CFP-10 (0.5 μg/ml) or ESAT-6 (5 μg/ml) (gift from Karin Weldingh, Statens Serum Institut, Denmark). PKH-26 dilution assays by flow cytometry (studied at 7 days of culture) were performed after CFP-10 exposure. The technical procedures of these assays have been detailed elsewhere.[24]

The cutoff value for a positive response was established with data from 21 control donors without LTBI and 24 patients with confirmed previous active TB infection (unpublished data), as described by Brock *et al*.[25] From analysis of receiver operating characteristic (ROC) curves, the optimal cutoff values for a diagnosis of LTBI were thymidine incorporation of 3.0 stimulation index (SI) for CFP-10 and 4.5 SI for ESAT-6, 4.6% proliferating cells for CFP-10 PKH-26 dilution, and 10.0 and 5.0 IFN-γ producing cells/10^6 cells for CFP-10 and ESAT-6 ELISPOT assays, respectively. All subjects underwent testing with the 3 tests CFP-10 antigen, and 33 patients underwent ESAT-6 antigen testing (17 in group I, 16 in group II).

As a positive control, stimulation with phytohemagglutinin (PHA; 5 μg/ml) (Murex Diagnostics, Paris, France) was used in ELISPOT testing. Spots were uncountable in most PHA stimulations and, in all cases, were more than 50 spots per well.

All experiments were performed with blinding to the results of previous blood tests, TST results, and the final diagnosis of the patients.

Statistical analysis

Results of in vitro blood tests were expressed graphically with means ± standard error of the mean (SEM) and proportions of positive results. Group data were compared by use of Mann–Whitney test for quantitative variables and χ^2 test (or Fisher's exact test when appropriate) for qualitative variables. For all statistical tests, a $p<0.05$ was considered significant. Statistical analysis involved the use of SAS 9.1 (SAS Institute Inc., Cary, NC).

Table 1 Characteristics of patients with immune-mediated inflammatory diseases (IMID) with (group II) or without (group I) latent tuberculosis infection (LTBI)

	Group I no LTBI	Group II LTBI
n	33	35
Age, mean (range), years	42.5 (17–82)	51 (31–79)
IMID (RA/SA/CD), no. of patients	11/10/12	18/15/2
Immunosuppressors (MTX/AZA/CT), no. of patients	16/6/18	15/1/11
Positive TST result (⩾10 mm), no of patients	0/33	30/35
Known size of TST result, no. of patients	23/33	27/35
⩽5 mm	13/23	3/27
6–10 mm	10/23	0/27
>10 mm	0/23	24/27

n, number of patients in each group; RA, rheumatoid arthritis; SA, spondylarthropathy; CD, Crohn's disease; MTX, methotrexate; AZA, azathioprine; CT, corticosteroids (equivalent of prednisone ⩽10 mg/day); TST, tuberculin skin test.

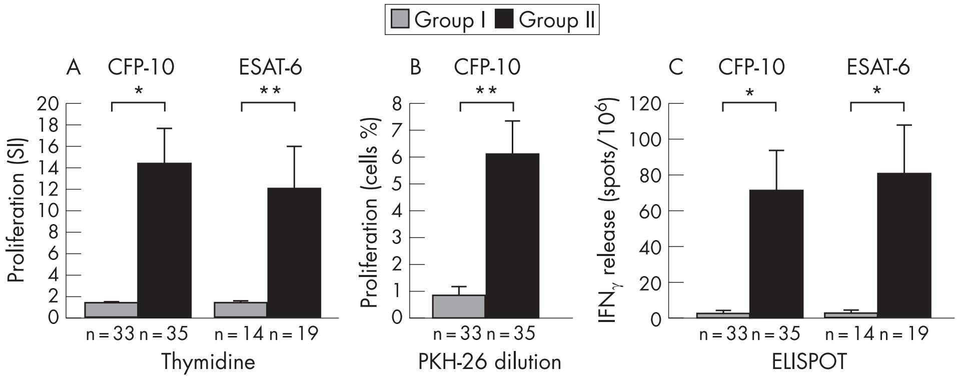

Figure 1 Responses to CFP-10 and ESAT-6 antigen testing in IMID patients with (group II, black boxes) or without (group I, diagonally lined boxes) latent tuberculosis infection. Results of thymidine incorporation, PKH-26 dilution and ELISPOT assays are reported in fig 1A,B,C, respectively and are expressed as mean $\pm$ standard error of the mean (SEM) stimulation index (SI), fraction of proliferating cells (%) and number of IFNγ-producing cells per 10^6 cells, respectively. Antigens tested are noted above the histograms, with the number of patients reported under each. Results between the two groups were compared for each assay and each antigen (*p<0.0001, **p<0.002).

RESULTS

Immune responses to CFP-10 and ESAT-6 in patients with IMID

The responses to the TB-specific antigens CFP-10 and ESAT-6 according to the 3 different assays are reported in fig 1. Because these antigens are specific to mycobacterium TB and are absent from BCG, responses to CFP-10 or ESAT-6, as expected, differed greatly between both groups, with some variations between the different tests (table 2). Notably, with the combined ESAT-6 and CFP-10 ELISPOT assay, 3/14 patients (21.4%) in group I and 17/19 (89.5%) in group II had positive results (table 2). Underlying IMID or associated immunosuppressive drug use did not affect the results (data not shown).

Comparison between in vitro blood assays and TST

Currently, no gold standard exists for LTBI diagnosis. In fact, to date, patients are considered as having LTBI mainly because of a positive TST result, although the TST can give false-negative and false-positive results, so our group II patients might include patients without real LTBI. Thus, to compare the performance of our in vitro tests with that of TST, we focused on the 13 patients in group II with confirmed LTBI based on previous primo-infection, previous TB with inadequate treatment or sequellar TB lesions on chest radiography as already mentioned, independent of TST result. Proliferative responses in the thymidine proliferation assay with CFP-10 or ESAT-6 for the 13 patients were strong (mean 18.9 (7.1) and 21.2 (9.9) SI, respectively). Only 2 out of these 13 patients (15.4%) had negative thymidine proliferation assay results (table 3). PKH-26 dilution assay for only CFP-10 gave more negative results than the other tests (8/13 (61.5%) patients with negative results). For ELISPOT assays, the 7 patients tested for both CFP-10 and ESAT-6 antigens had a positive result (table 4).

Among these 13 patients with confirmed LTBI independent of TST result, 5 (38.5%) had a negative TST result ($\leqslant$10 mm) (table 3). All 5 had positive test results on thymidine proliferation assay with CFP-10 (mean 18.9 (8.4) SI). The 2 patients with negative TST who have been tested with CFP-10 and ESAT-6 in ELISPOT assays had positive results (table 4).

Table 2 Positive results obtained with in vitro assays in group I and II

Antigen	Test	Group I no LTBI	Group II LTBI	P
CFP-10	Thymidine	1/33 (3)*	23/35 (65.7)	<0.0001
	PKH-26	1/33 (3)*	16/35 (45.7)	<0.0001
	ELISPOT	1/32 (3.1)*	18/35 (51.4)	<0.0001
ESAT-6	Thymidine	0/14 (0)	10/19 (52.6)	0.0014
	ELISPOT	2/14 (14.3)	16/19 (84.2)	<0.0001
CFP10 + ESAT-6	ELISPOT	3/14 (21.4)	17/19 (89.5)	<0.0001

*The 3 patients with positive results are different.
Results are expressed as number of patients (%).

Significance of an intermediate TST result

Ten of 23 patients with an exact size of TST induration were included in group I (patients without LTBI) and had a TST result between 6 and 10 mm. These patients with intermediate TST results, according to the recent French recommendations and to most worldwide recommendations, would have been considered as having LTBI and thus included in group II only because of the results of the TST.

We compared the in vitro assay results among these 10 patients who had a TST result between 6 and 10 mm, group I patients who had a TST result $\leqslant$5 mm, and group II patients (the LTBI group). The results of in vitro tests for the 10 patients were found not to be different from those for patients with a TST result $\leqslant$5 mm (p>0.3 for each assay) and much lower than those for group II patients—the LTBI group (p<0.002, p$\leqslant$0.003 and p<0.05 for CFP-10 thymidine and CFP-10 and ESAT-6 ELISPOT assays, respectively) (fig 2). Likewise, we observed no difference between patients with a TST result between 6 and 10 mm and those with a result $\leqslant$5 mm in terms of the number of patients testing positive for each assay (table 5).

DISCUSSION

Commercial in vitro T-cell based assays for IFNγ-release (T-SPOT TB and QFT-G) have been validated in unselected and HIV-positive populations,[12] but, despite its widely recognised limitations, the TST remains the classical biological method for identifying TB infection before anti-TNF therapy. To date, only a few reports of the use of in vitro tests in IMID have been

Table 3 Results of CFP-10 thymidine incorporation among patients with certain latent tuberculosis infection independent of the tuberculin skin test (TST) result

Patient	TST result	CFP-10 thymidine incorporation
Patient 1	Neg	Pos (3.0)
Patient 2	Neg	Pos (6.9)
Patient 3	Neg	Pos (6.2)
Patient 4	Neg	Pos (42.6)
Patient 5	Neg	Pos (35.7)
Patient 6	Pos	Pos (28.1)
Patient 7	Pos	Pos (90.1)
Patient 8	Pos	Pos (3.1)
Patient 9	Pos	Neg (1.8)
Patient 10	Pos	Pos (15.4)
Patient 11	Pos	Neg (1.3)
Patient 12	Pos	Pos (3.0)
Patient 13	Pos	Pos (8.1)
Total	**8/13 (61.5%)**	**11/13 (84.6%)**

TST is expressed as positive with a threshold of 10 mm. Results of each test for each subject are reported as Pos (positive) or Neg (negative) (exact value). Results for thymidine assay are expressed in the stimulation index (SI).

Table 4 Results for ELISPOT assays among patients with certain latent tuberculosis infection independent of the TST result

Patient	TST result	CFP-10 ELISPOT	ESAT-6 ELISPOT	CFP-10 or ESAT-6 ELISPOT
Patient 3	Neg	Pos (30)	Pos (25)	Pos
Patient 4	Neg	Pos (76.7)	Neg (0)	Pos
Patient 6	Pos	Neg (1.7)	Pos (8.7)	Pos
Patient 7	Pos	Neg (5)	Pos (33.3)	Pos
Patient 8	Pos	Pos (11.7)	Pos (8.5)	Pos
Patient 12	Pos	Neg (0)	Pos (366.5)	Pos
Patient 13	Pos	Pos (18.3)	Pos (116.6)	Pos
Total	**8/13 (61.5%)**	**4/7 (57.1%)**	**6/7 (85.7%)**	**7/7 (100%)**

Patients tested for both antigens (CFP-10 and ESAT-6) were analysed (n = 7). TST is expressed as positive with a threshold of 10 mm. Results of each test for each subject are reported as Pos (positive) or Neg (negative) (exact value). Results for ELISPOT are expressed as the number of IFNγ-producing cells/10^6 cells.

published.[24 26 28] In our study, involving 68 patients with IMID, we investigated an IFNγ-release ELISPOT assay, which is equivalent to the commercial assay with the combined use of CFP-10 and ESAT-6 antigens, and this test platform can also provide useful additional data about the specific mycobacterial immune response. Indeed, proliferation assays for thymidine incorporation and PKH-26 dilution investigate the central memory response, whereas it is generally believed that the IFNγ-release test assesses the effector memory response. Notably, we recently reported on a prospective *ex vivo* study demonstrating that TNF blocker treatments impaired IFNγ release in response to *M. tuberculosis* antigens, while proliferative responses were preserved.[24]

In our study, IMID patients with LTBI had significantly higher values for all tests than IMID patients without LTBI. Among the 14 patients in group I who underwent proliferation and ELISPOT tests against both CFP-10 and ESAT-6, 3 (21.4%) had a positive result with at least 1 of the in vitro blood assays but a negative TST result. This finding can be interpreted either as a better sensitivity of in vitro tests as compared with TST and other signs of LTBI or as false-positive results with the latter assays.

Comparison of sensitivity and specificity of the assays with that of TST is impossible because the TST result is used to define LTBI in most cases, and no gold standard exists to define LTBI. Thus, we focused on the 13 patients with certain LTBI based on clinical or radiographic findings, independent of the TST result. Overall, these patients had a positive result with in vitro assays, and only 2 (15%) had a negative result, whereas 5 (38.5%) had a negative TST result, which argues for the better sensitivity of in vitro assays than the TST for LTBI in IMID. Notably, the 2 patients with negative results with in vitro assays were not tested for ESAT-6 antigen level, and it is possible that these patients had an inability to respond to CFP-10 antigen because of HLA system restriction. Moreover, the 5 patients with negative TST results all had a positive anti-CFP-10 response with thymidine incorporation. As T-SPOT TB is performed using CFP-10 and ESAT-6 antigens simultaneously and because of the variability of the antigen presentation related to the HLA system of each patient, only patients with certain LTBI and who have been tested for these both antigens have been analysed. Interestingly, all 7 patients in this situation had positive ELISPOT assays considering the results of CFP-10 or ESAT-6, whereas 2 had negative TST (table 4). Thus, the sensitivity of in vitro tests to diagnose LTBI is higher than that of TST, and in vitro blood tests can be helpful to diagnose LTBI in patients with IMID, even in cases of immunodepression. Notably, all patients responded to PHA, which argues for a selective diminished response to PPD and for an absence of profound anergy in our IMID patients, as has already been observed in RA patients.[29]

In group II (the LTBI group), in vitro blood assays gave positive results at a frequency between 45.7% and 84.2%. ELISPOT assay was efficient to detect LTBI, especially when CFP-10 and ESAT-6 were combined, with a better sensitivity than proliferation assays. Nevertheless, among the 17 patients of group II with negative results on anti-CFP10 ELISPOT assay, 7 had a positive result of CFP-10 thymidine incorporation assay. Among the 3/19 patients with negative results on ESAT-6 ELISPOT assay, 1 had a positive result of ESAT-6 thymidine incorporation assay. These data suggest that a combination of assays, such as ELISPOT and thymidine assays, could be helpful in particular situations. Further investigations are warranted to establish the utility of thymidine proliferation assay, taking into account its longer technical procedure. The proportion of patients from group II with positive results from in vitro tests

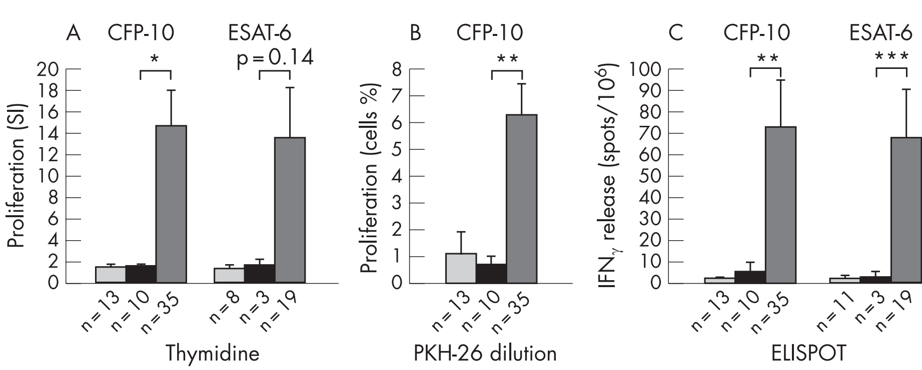

Figure 2 Results of in vitro blood tests in patients without latent tuberculosis infection (LTBI) and tuberculin skin test (TST) ⩽5 mm (diagonally lined boxes), those without evidence of LTBI but a TST result between 6 and 10 mm (black boxes) and those with LTBI (corresponding to the group II) (horizontally lined boxes). Results of thymidine incorporation, PKH-26 dilution and ELISPOT assays are reported in fig 1A,B,C, respectively, and expressed as mean ± standard error of the mean (SEM) stimulation index (SI), fraction of proliferating cells (%) and number of IFNγ-producing cells per 10^6 cells, respectively. Antigens tested for each test are noted on top of the histograms, with the number of patients reported under each. Statistical analysis compared the group with intermediate TST (6–10 mm) with the two others (*$p<0.002$; *$p \leqslant 0.003$; **$p<0.05$).

Table 5 Comparison of positive results of in vitro assays among patients without latent tuberculosis infection (LTBI) and tuberculin skin test (TST) results $\leqslant 5$ mm, those without evidence of LTBI except a TST result between 6 and 10 mm (intermediate TST) and those with LTBI (corresponding to group II)

Antigen	Test	Patients without LTBI and TST $\leqslant 5$ mm	Patients with TST 6–10 mm	Patients with LTBI Group II	P[1]	P[2]
CFP-10	Thymidine	1/13 (7.7)*	0/10 (0)	23/35 (65.7)	0.99	<0.0001
	PKH-26	1/13 (7.7)*	0/10 (0)	16/35 (45.7)	0.99	0.008
	ELISPOT	0/13 (0)	1/10 (10)	18/35 (51.4)	0.43	0.03
ESAT-6	Thymidine	0/8 (0)	0/3 (0)	10/19 (52.6)	1.0	0.22
	ELISPOT	1/8 (12.5)	1/3 (33.4)	16/19 (84.2)	0.50	0.12

*The 2 patients with positive results are different.
P[1], patients with intermediate TST result (6–10 mm) compared with patients without LTBI and TST result $\leqslant 5$ mm. P[2], patients with intermediate TST result (6–10 mm) compared with patients with LTBI.

shows that results from these tests are not in perfect agreement with conventional recommendations because patients were included in the LTBI group mainly on the basis of TST result, which was positive in 30 patients of group II and could correspond to false-positive in a BCG-vaccinated population.

We demonstrated the high frequency of false-positive results of the TST result between 6 and 10 mm if a threshold of 5 mm is chosen, taking into account as the gold standard results of in vitro tests in patients with intermediate TST results. In France, before July 2005, the TST threshold in patients with IMID was 10 mm, which differed from the threshold of most other countries (5 mm) and was justified by the nearly 100% vaccination with BCG.[8 9] According to a prospective study of the RATIO group, 4 of the first 13 cases of active TB treated with TNF blockers had a TST result between 6 and 10 mm ([10] and personal communication). This observation led to a reduction in the TST threshold from 10 to 5 mm, which is the cutoff largely used in other countries to detect LTBI before anti-TNFα therapy.[4 6 30] But the risk was in giving excess antibiotics to patients with intermediate TST results simply because of previous BCG vaccination. To investigate the biological relevance of this recommendation, we analysed 10 patients with an intermediate TST result who would have received antibiotics if they began anti-TNFα therapy after July 2005. The in vitro assay results for these patients differed significantly from those of patients with LTBI (group II) and were similar to those with a TST result $\leqslant 5$ mm according to their quantitative results. Only 2 had positive results with the CFP-10+ESAT-6 ELISPOT assays, and none had positive results with the thymidine proliferation and PKH-26 dilution assays. This result suggests that this change in cutoff level has led to a decrease in the specificity of the TST. Moreover, using ROC curve analysis, we have determined the optimal cutoff values of TST for a diagnosis of LTBI, considering that positive CFP-10 thymidine incorporation was the gold standard of LTBI. Interestingly, we have found that the optimal TST cutoff was 9 or 10 mm using CFP-10 thymidine incorporation assay, suggesting also that the 5-mm cutoff is too low and can lead to a diagnosis of LTBI in excess (data not shown). The logical consequence is the possibility of inappropriate antibiotics prescription, as has been suggested by others,[31] and the risk of antibiotic toxicity.[13–15] Thus, the in vitro tests probably have better specificity than TST in IMID for LTBI diagnosis, especially with the 5-mm cutoff for TST. Notably, these 10 patients who have not been treated by antibiotics have not developed further active TB infection with anti-TNF therapy.

In the general population, IFNγ-release assays offer a more accurate approach than TST for identifying individuals with LTBI. Indeed, among recent TB contacts and among HIV-positive patients, such test results identified *M. tuberculosis* exposure better than did the TST.[12 32 33] Except for the HIV-positive population, patients with immune deficiency have not undergone exploration with in vitro blood assays. Piana *et al* found among 96 patients with haematological disorders and negative TST results 34 (35%) with a positive T-SPOT TB result.[21] Richeldi *et al* also detected a subclinical active TB infection in a patient with Crohn's disease with a false-negative TST result,[26] and more recently, Efthimiou *et al* reported two cases of LTBI with a negative TST result and a positive QFT-G test result before anti-TNF therapy.[27] Our results and these data suggest a better sensitivity and specificity of in vitro blood tests than the TST in IMID. These results should be confirmed in larger studies.

In conclusion, new in vitro T-cell based assays investigating immune-specific anti-TB response can benefit patients with IMID despite immunodepression state and might be a powerful new tool in the diagnosis of LTBI in the IMID population. In our study, compared with the TST, in vitro tests had both a better sensitivity, with positive results in 5 patients with certain LTBI and negative TST results, and a better specificity, with negative results in most patients having intermediate TST results (6–10 mm) as the only criteria of LTBI. These tests, and especially commercial kits, need to be evaluated further in IMID patients by comparison with TST results.

Prophylactic anti-TB antibiotics before anti-TNF therapy are necessary in the presence of certain clinical and/or radiographic findings of LTBI. In the absence of these findings, specific anti-TB in vitro blood tests could replace the TST for deciding prophylactic antibiotic treatment.

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